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# **Variants in autophagy genes trigger susceptibility to both Crohn's disease and *H. pylori* infection**

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*Helicobacter pylori* (*H. pylori*) is a Gram-negative, microaerophilic bacterium that selectively colonizes the stomachs of half the world's human population. *H. pylori* is the etiological agent of several gastric diseases that include chronic gastritis and peptic ulcers. Long-term chronic inflammation subsequently increases the risk for the development of mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer <sup>1, 2</sup>. During a well-choreographed interaction between the bacteria and the host gastric epithelium, *H. pylori* infection initiates an immune response that leads to a massive infiltration of inflammatory cells <sup>1</sup>. An evolutionarily-conserved cellular mechanism, autophagy, functions as an innate defense lysosomal pathway in response to infection to degrade intracellular microorganisms attempting to establish a replicative niche in the host epithelial cell cytoplasm <sup>3</sup>. Unfortunately, *H. pylori* utilizes a novel escape mechanism to evade lysosomal destruction in host epithelial cells that subsequently supports chronic infection. A study in this month's issue of *Gastroenterology* identifies, for the first time, an autophagy gene as a candidate for host susceptibility to *H. pylori* infection and disease progression <sup>4</sup>.

### **The *H. pylori* vacuolating cytotoxin (VacA)**

Vacuolating cytotoxin (VacA) is an important virulence factor for *H. pylori* disease pathogenesis. The VacA gene encodes a 96-kDa precursor protein that is secreted and cleaved into an 88-kDa mature protein and a 10.5-kDa passenger domain <sup>1</sup>. The mature 88-kDa toxin can undergo further proteolytic cleavage to yield the p33 and p50 fragments which represent the VacA functional domains that are required for toxin activity <sup>5</sup>. The most well recognized effect of VacA intoxication of mammalian cells is

the induction of vacuolation <sup>6</sup>. The toxin generates the formation of membrane channels by being secreted as monomers that oligomerize at the host plasma membrane <sup>6</sup>. Glycosylphosphatidylinositol anchored proteins enriched early endosomal compartments or GEECs are involved in endocytosis of VacA <sup>7</sup>. VacA then traffics to lysosomal compartments where the toxin induces vacuolation via a mechanism dependent on GTPase Rab7 <sup>8, 9</sup>, dynamin <sup>10</sup> and syntaxin <sup>7 11</sup>. In addition to vacuolation, VacA intoxication has detrimental effects that include inhibition of T cell proliferation <sup>12</sup> and the induction of apoptosis <sup>6</sup>, increased cellular permeability <sup>8</sup> and autophagy within gastric epithelial cells <sup>3, 9</sup>. Initial studies demonstrated that *H. pylori* invades gastric epithelial cells and resides within vacuoles <sup>13</sup>. Later experiments then showed that these vacuoles were associated with the autophagy pathway and attributed to bacterial intracellular survival of *H. pylori* within gastric epithelial cells <sup>3, 9</sup>.

### **Autophagy in response to *H. pylori* VacA and persistent bacterial infection**

In recent years autophagy has been recognized as being of central importance for the maintenance of cell homeostasis and survival but also for the regulation of inflammation and for bacterial defense at body surfaces <sup>14</sup>. Although *H. pylori* is generally considered to be a noninvasive pathogen, emerging evidence demonstrates that the bacteria also invade and replicate within autophagosomes of macrophages, dendritic and epithelial cells <sup>3, 9, 12, 15</sup>. In view of the observation that *H. pylori* infection induces autophagy within epithelial cells <sup>3, 9</sup>, the question then remains as to how *H. pylori* are allowed to replicate within the autophagosome and evade lysosomal-induced degradation.

Raju and co-workers <sup>4</sup> recently reinforced that VacA, independent of the bacteria, alters the degradative capacity of the endocytic pathway. Using gastric cancer epithelial AGS cells treated with culture supernatants from VacA-positive *H. pylori* the investigators show that although VacA intoxication induces autophagosome-lysosomal fusion cathepsin D levels were negligible <sup>4</sup>. Cathepsin D is a key hydrolase necessary for lysosomal-induced degradation. Therefore, lack of cathepsin D would alternately disrupt autophagic degradative process and thus offer a plausible mechanism by which *H. pylori* replicate within autophagosomes.

The investigators then extended the in vitro findings by studying the status of autophagy within human gastric biopsies collected from patients infected with *H. pylori*. The signaling adaptor p62 is a multidomainprotein implicated in the activation of the transcription factor NF- $\kappa$ B, apoptosis and autophagy. Dysfunctional autophagy leads to an accumulation of p62 that contributes directly to tumorigenesis <sup>16</sup>. Indeed, gastric tissue sections collected from patients infected with the toxigenic s1m1 VacA-producing *H. pylori* strain showed increased accumulation of p62 expression in the foveolar cells of the gastric epithelium <sup>4</sup>. However, the most interesting discovery that was made by Raju et al. <sup>4</sup> was that *H. pylori* infection was increased in individuals harboring a polymorphism in the *ATG16L1* autophagy gene.

### **The Crohn's variant of ATG16L1 as it relates to *H. pylori* susceptibility**

In autophagosomes, structures that need to be eliminated are sequestered into double-membrane-enclosed vesicles and delivered to lysosomes for final degradation. Autophagosome assembly involves activation of beclin-1 and conjugation of ATG12-

ATG5 that is catalyzed by ATG7 and ATG10<sup>17</sup>. The resulting ATG5-ATG12 conjugate is stabilized by a non-covalent complex with ATG16L1 that mediates, in addition to ATG7 and ATG3, the conversion of LC3B-I to LC3B-II by lipidation with phosphatidylethanolamine<sup>17</sup>. Interestingly, a variant of the ATG16L1 gene (rs2241880, causing amino acid substitution T300A) has been associated with the risk to develop Crohn's disease<sup>18, 19</sup>. Functional studies have shown that ATG16L1 and autophagy is critically involved in host defense against intracellular pathogens, such as *Listeria monocytogenes* or *Salmonella typhimurium*<sup>20, 21</sup>. Dysfunction of ATG16L1 has been implicated not only in defective autophagy and, consequently, bacterial handling, but also in altered gene/protein expression patterns in intestinal cells<sup>20, 22</sup>. The presence of the Crohn's disease associated T300A polymorphism within the ATG16L1 gene leads to the development of dysmorphic and dysfunctional Paneth cells in the intestine of Crohn's disease patients<sup>22</sup>.

In their recent manuscript in this month's issue of *Gastroenterology*, Raju and co-workers demonstrate that the T300A variant of *ATG16L1*, increases the susceptibility to *H. pylori* infection and partially prevents the VacA induced induction of autophagy. This indeed indicates that the process of autophagy has a protective role for bacterial invasion not only in the ileum or colon but also in the gastric mucosa. Autophagy under this vies appears to be a very basic and broad innate defense mechanism not only to eliminate whole bacteria but also their toxins during infection. However, the prolonged exposure to VacA leads to an impairment of autophagy and subsequently a failure to clear *H. pylori* infection. Thus, chronic infection and subsequent accumulation of toxic material may aggravate the process.

In conclusion, the study by Raju et al.<sup>4</sup> advances our current knowledge of VacA as an important pathogenic factor for *H. pylori*. **Figure 1** illustrates the process of increased *H. pylori* susceptibility and disease progression in response to the *ATG16L1* variant associated with the risk to develop Crohn's disease. During the initial stages of *H. pylori* infection VacA initiates autophagy. The data presented by Raju et al.<sup>4</sup> collectively suggests that the bacterial load and level of VacA intoxication may be driving factors that determine the overall susceptibility to chronic infection and development of disease. Based on this notion, during initial *H. pylori* exposure, if the bacterial load and VacA intoxication is low one would expect that host cell autophagy reduces the effects of the toxin and clears infection (**Figure 1A**). However, the current study also elegantly demonstrates that in individuals carrying the *ATG16L1* risk allele there is a reduced autophagic response. Therefore, levels of low bacterial load and VacA toxin become enhanced, subsequently increasing the susceptibility of infection and disease progression (**Figure 1B**). Prolonged exposure of VacA intoxication, that would commonly be associated with chronic infection, then results in the disruption of the autophagic degradative process that further exacerbated bacterial infection and disease by promoting *H. pylori* intracellular survival (**Figure 1B**). Therapeutic options for the treatment of not only Crohn's disease patients but also *H. pylori* infection may arise from insights into how impaired autophagy can be restored under these conditions.



## Figure Legend

**Figure 1: A model for the process of increased *H. pylori* susceptibility and disease progression in response to *ATG16L1* variants associated with Crohn's disease.** (A) During initial *H. pylori* exposure, if the bacterial load and VacA intoxication is low host cell autophagy is effective in reducing the effects of the toxin and clearing the infection. (B) Individuals carrying the *ATG16L1* risk allele have a reduced autophagic response. Levels of bacterial load and VacA toxin become enhanced subsequently increasing the susceptibility of infection and disease progression. Prolonged exposure of VacA results in the disruption of the autophagic degradative process that further exacerbates bacterial infection and disease by promoting *H. pylori* intracellular survival.

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